

SEX DIFFERENCES IN BLADDER DYSFUNCTION IN RESPONSE TO ENTERIC NEURONAL NFKB OVERACTIVATION AND EXPERIMENTALLY INDUCED COLITIS IN MICE.

Hypothesis / aims of study

There is an ever increasing interest in cross talk between visceral organs that is believed to result in symptoms in multiple organs in conditions such as chronic pelvic pain, irritable bowel syndrome (IBS) and interstitial cystitis (IC). Many patients with one of these conditions exhibit symptoms commonly found in another one of these conditions. In experimental models, animals with induced colitis demonstrate urinary bladder dysfunction. This includes increased urinary frequency and abnormalities in bladder smooth muscle response to cholinergic and electric field stimulation (EFS). Nuclear factor kappa B (NFkB) is a major mediator of the inflammatory response in inflammatory bowel disease (IBD) and IBS. During acute inflammation in the colon, epithelial NFkB plays a vital role in protecting mucosal integrity. However, during chronic inflammation NFkB in immune and smooth muscle cells mediates the inflammatory response. The aim of the present study was to determine the effect of increased NFkB signalling specifically in enteric neurons on bladder contractions in the presence or absence of experimentally induced colitis.

Study design, materials and methods

Calretinin-Cre-ERT2-IKK2CA/tdTomato transgenic (TG) mice were generated by crossbreeding a conditional stop-floxed mouse line carrying a constitutively active form of inhibitor of nuclear factor kappa-B kinase (IKK2CA) with Rosa26-Stop Floxed-tdTomato reporter mice and then with Calretinin-Cre-ERT2 mice. At two months of age, the mice were treated for 3 days with 100 mg/kg tamoxifen causing IKK2CA expression and continuous NFkB activation specifically in the calretinin-expressing enteric neuronal cells. One month later, the mice were treated with vehicle or 2.5% dextran sulfate sodium (DSS) for 7 days to induce colitis. Full thickness urinary bladder strips were mounted in organ baths and induced to contract with buffer containing 120 mM KCl and in response to electric field stimulation (nerve mediated contraction). After performing an initial frequency response curve (12 volts, 1 ms, 2-30 hz), the bladder strips were either incubated with 10 uM atropine to inhibit muscarinic receptors or 30 uM alpha, beta methylene ATP to desensitize purinergic receptors and a second frequency response curve was recorded.

Results

At the time of colitis induction, around 50% of colonic enteric neurons determined by immunohistochemical staining with anti-HuD antibody were labelled by the tdTomato reporter confirming IKK2CA expression and NFkB activation in calretinin-expressing neurons. There was no significant effect on food and water intake or colonic motility in TG mice compared to littermate wild-type (WT) mice. After induction of colitis with DSS for 7 days the weight loss, bleeding and mucosal damage were less in TG mice than WT mice.

Even though the bladder contractile response to high potassium stimulation was similar between sexes in both WT and TG mice, the EFS induced (nerve mediated) maximal contraction was greater in WT males than in WT females. In TG mice, the maximal nerve mediated contraction was higher in males than in females as a percentage of the potassium induced response. Bladder strips from WT female mice had a significantly greater atropine resistant (purinergic) component of nerve mediated contraction compared to WT male mice, but this was not found in TG mice. The cholinergic component of nerve mediated contraction (remaining contraction following desensitization with ATP) was not different between male and female mice (WT or TG). Nerve mediated contraction is nearly entirely mediated by acetylcholine and ATP because blocking muscarinic receptors and desensitizing purinergic P2X receptors nearly eliminated EFS induced contraction.

Acute colitis significantly reduced KCl and nerve mediated contraction in bladder strips from WT female and male mice and TG female mice. Constitutive activation of neuronal NFkB preserved the nerve mediated and KCl induced contractile response in male mice. Acute colitis increased the purinergic component of nerve mediated contractions in male WT mice only.

Interpretation of results

Significant sex related differences exist in the nerve mediated bladder contractile response. Bladder strips from male mice have larger nerve induced contractions and a smaller purinergic component of nerve mediated contractions than female mice. This finding could be due to anatomical or possibly hormonal differences between female and male mice. Constitutive activation of NFkB signalling in enteric (and perhaps bladder) neurons partially blocks the reduction in contraction of male but not female bladder strips induced by DSS treatment. This effect may be because DSS treatment in TG male mice significantly increases the purinergic component of nerve mediated contractions but not in females. The purinergic component of nerve mediated contraction also increases in humans with IC (1) suggesting similar physiological alterations.

Concluding message

Experimentally induced colitis causes neurogenic inflammation in the bladder (2) along with decreased bladder contraction. Neuronal NFkB signalling rescued the decreased bladder contraction due to colitis perhaps through purinergic regulation in males. Neurogenic inflammation in the bladder may cause a larger degree of bladder dysfunction in female than in male mice perhaps because females have a greater purinergic component of nerve mediated bladder contraction which does not further increase due to colitis.

References

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Disclosures

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